

A Phase I Trial of Intranasal Moli1901 for Cystic Fibrosis*

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Background: The peptide drug Moli1901 activates an alternative chloride channel that is present in cystic fibrosis (CF) nasal and airway epithelia. Doing so bypasses the dysfunctional CF transmembrane regulator.

Study objective: To determine whether intranasal Moli1901 is safe, tolerable, and will induce chloride transport in healthy volunteers and CF subjects.

Design: A single-blind (to the participant), randomized, placebo-controlled, dose-escalation study of intranasal Moli1901 was performed in four healthy non-CF participants and four participants with CF. Drug or placebo was administered by intranasal superfusion, and nasal potential difference responses were continuously monitored during sequential dose escalations at 1-min intervals from 0.01 through 10 $\mu\text{mol/L}$.

Results: Neither Moli1901 nor placebo were associated with visible changes such as edema, erythema, drainage, secretions, or ulcer formation. No elevations in lactate dehydrogenase, albumin, or cell counts were observed in nasal lavage fluid after administration. No clinically significant changes in FEV₁ or other toxicity parameters occurred. Changes in the nasal potential difference (NPD) induced by chloride-free, amiloride-containing Ringers solution and by subsequent superfusion with the same solution plus 10 $\mu\text{mol/L}$ isoproterenol were consistent with both an acute and a sustained change in chloride transport in response to Moli1901. A similar analysis of NPD in the four CF participants demonstrated an acute response that resolved more quickly. A dose-response relationship to Moli1901 was observed in non-CF participants, but a greater range of variability within the CF participants contributed to the lack of a clear dose-response relationship in this group.

Conclusion: Moli1901 stimulates chloride transport in normal and CF nasal epithelia *in vivo*, but may have a shorter duration of action in CF participants. (CHEST 2004; 125:143-149)

Key words: calcium; chloride transport; nasal potential difference; peptide

Abbreviations: cAMP = dibutyl adenosine 3',5'-cyclic monophosphate; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane regulator; I_{sc} = short circuit current; LDH = lactate dehydrogenase; mV = millivolt; NPD = nasal potential difference

Cystic fibrosis (CF) is one of the most common life-shortening autosomal recessive inherited disorders in the white population. Mutations in the

gene that encodes the CF transmembrane conductance regulator (CFTR) disrupt dibutyl adenosine 3',5'-cyclic monophosphate (cAMP)-mediated chloride transport in exocrine and secretory epithelial tissues. CF is a systemic disorder; however, most of the morbidity and mortality in CF occurs secondary to progressive pulmonary infection, inflammation, and airway obstruction. Restoration of chloride transport by an alternative pathway such as calcium-activated chloride channels might compensate for absent or reduced CFTR function in airway epithelia.

Compound Moli1901 (formerly known as 2622U90 or duramycin) is a stable 19-residue polycyclic peptide that increases chloride transport and fluid secretion when applied to the apical surface of airway epithelium.^{1,2} This peptide, which is derived from *Streptomyces cinnamoneum*, interacts with

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phospholipids present in plasma and organelle membranes,³ where it elevates intracellular calcium and in turn activates an alternative chloride conductance pathway.^{4,5} Preclinical toxicology and pharmacokinetic studies have defined a therapeutic window in which aerosol doses were found to be safe and provided an estimate of a remarkably long half-life of 64 days in the rat airway epithelium.⁶ A preliminary study⁷ in dogs suggested a half-life of 25 days. Compound Moli1901 is excreted in the feces nearly completely, presumably by swallowing sloughed epithelial cells after they are passed along the mucociliary escalator to the pharynx.^{6,7} Thus the prolonged duration of action after single-dose administration^{1,2} may possibly be accounted for by the half-life of airway epithelial cells in mature lungs.

The nasal epithelium resembles bronchial epithelium both in morphologic and physiologic properties.⁸ Measurements of the transepithelial potential difference across the inferior turbinate are reflective of the properties of bronchial epithelium.⁸ Initial dose escalation and safety studies of Moli1901 were therefore undertaken by intranasal superfusion in order to assess possible toxicity and to measure ion transport in a relatively safe location in the respiratory tract. The objectives of the study were to test the hypothesis that Moli1901 induces chloride secretion, establishes a dose-response relationship, determines the functional duration of action, and evaluates safety.

MATERIALS AND METHODS

Study Design

This was a phase I trial that was randomized, placebo controlled, and single blind to the participant. The study protocol was approved by the Johns Hopkins University School of Medicine Joint Committee on Clinical Investigation (institutional review board) and the Johns Hopkins General Clinical Research Center, and was performed in the Johns Hopkins Hospital Outpatient General Clinical Research Center. Participants gave informed consent.

Participant Characteristics

Healthy, non-CF participants 18 to 40 years of age were recruited locally. Exclusion criteria included a history of pulmonary disease, acute upper respiratory tract infection within the previous month, acute rhinitis, a nasal-septal defect or nasal polyps, pregnancy, or a history of atopy, hay fever, asthma, or chronic rhinitis. Additional exclusion criteria included any clinically significant liver, renal, cardiac, neurologic, hematologic, or GI disease, or smoking within past 6 months.

Participants with CF who met inclusion criteria were of both genders and ≥ 18 years old. The diagnosis of CF was established by genotype or elevated sweat electrolytes (> 60 mEq/L). An $FEV_1 > 60\%$ was required. Other exclusion criteria were identical to non-CF participants except for the additional exclusion criteria of a history of bronchospasm or surgical repair of sinusitis within the previous 6 weeks.

Safety Parameters

Safety was evaluated before and after treatment by physical examinations, vital signs, laboratory examinations (blood chemistry and hematology), ECG, nasal exams, nasal lavage, spirometry, and adverse experience assessments. Study personnel measured pulse rate and BP immediately following the completion of the superfusion of each dose. Nasal exam was scored (0 = none, 1 = mild, 2 = moderate, 3 = severe) for inflammation, edema, erythema, fissure, ulcer, polyps, drainage, and visible sinuses. Nasal lavage was performed on subjects in both nostrils after the highest dose had been superfused. Five milliliters of saline solution (0.9% NaCl) were gently injected in 1-mL increments into the vestibule. The subject expelled the fluid by gentle blowing into a collecting tube. The fluid was evaluated for RBC count, total WBC count and differential, albumin, and lactate dehydrogenase (LDH). Spirometry was performed in the pulmonary function laboratory according to laboratory standards.

Nasal Potential Difference

Nasal potential difference (NPD) was recorded from a high-impedance voltmeter (World Precision Instruments; Sarasota, FL) connected with an in-line Ag/Ag Cl electrode (World Precision Instruments) to a double-barreled exploring bridge (polyethylene-50 tubing) and to a subcutaneous bridge consisting of a 22-gauge butterfly needle filled with sterile Ringers solution. The protocol utilized was a modification of Knowles et al⁸. The area under the inferior nasal turbinate that provided the maximal baseline NPD was located during superfusion with sterile Ringers solution at a rate of 0.5 mL/min. On reaching a stable baseline potential difference, the area was then perfused with 100 $\mu\text{mol/L}$ amiloride in Ringers solution at 5 mL/min until a stable value was recorded (again at least 2 min). The difference between this final value and the basal NPD was called *amiloride-sensitive potential difference*. The perfusate was then changed to 100 $\mu\text{mol/L}$ amiloride in a chloride-free, gluconate-substituted Ringers solution, pH 7.4. The superfusion was continued at 5 mL/min for at least 2 min until a stable value was reached. The difference between this final value and the beginning of the chloride-free superfusion was called *low-chloride response*. The perfusate was then switched to 10 $\mu\text{mol/L}$ isoproterenol in the chloride-free/amiloride solution at 5 mL/min for at least 2 min until a stable value was again reached. The difference between this value and the value at initiation of the beginning of values in isoproterenol superfusion formed the isoproterenol response. All solutions were at room temperature, and freshly diluted isoproterenol was used for each procedure.

After establishing the baseline NPD characteristics and allowing a recovery period of at least 1 h, the vehicle and Moli1901 challenges were begun. Each participant was randomized to receive Moli1901 in one naris and vehicle in the other. Prior to administration of Moli1901 or vehicle, the baseline Ringers, amiloride, and chloride-free/amiloride superfusions were repeated. The isoproterenol response was not repeated, as the goal of this phase of the experiment was to determine whether Moli1901 stimulated chloride transport beyond what was observed with gluconate replacement. Escalating doses of vehicle (100 $\mu\text{mol/L}$ amiloride in chloride-free, gluconate-substituted Ringers, pH 7.4) or escalating doses of Moli1901 (in the vehicle solution) were applied sequentially by superfusion of 3 mL total at 3 mL/min through one barrel of the double-barreled exploring bridge. The other catheter continued to be very slowly perfused with the chloride-free/amiloride solution at only 0.05 mL/min. Nasal examination was performed between doses. A total of seven doses of vehicle or Moli1901 were applied sequentially to one naris before switching to the other one.

RESULTS

Subjects

Four non-CF (one woman and three men) and four CF participants (three women and one man) enrolled and completed the study. One CF subject presented at screening with an elevated serum glucose level that was subsequently evaluated and treated.

Safety

No adverse events related to Moli1901 occurred during this study. There was no change in FEV₁ after exposure to Moli1901 in either group. All eight subjects had no statistically significant change in any safety parameter after exposure to Moli1901.

All subjects were evaluated for anomalies of the nasal epithelium and judged to be suitable for inclusion in the study. Both control and drug-treated nares showed mild-to-moderate edema (n = 5), erythema (n = 4), and mild bleeding (n = 1) during treatment and/or after treatment. These findings were judged to be related to the conduct of the study, *eg*, to multiple reinsertions of the catheters in the nostril mandated by the need to directly inspect the nasal turbinates after each dose, rather than the nature of the superfusate.

There was no indication that superfusion with Moli1901 produced ulcer, edema, erythema, drainage, or secretions. The lavage components of primary interest, albumin and LDH levels, were collected before and after treatment for seven of eight subjects, due to a laboratory error. No evidence of increased cell permeability was noted as measured by albumin and LDH in the nasal lavage fluids.

Minor adverse events reported by the non-CF group included nasal congestion, headache, erythema on one arm and a GI illness (n = 1 each). All resolved during the course of the study.

NPD IN NON-CF AND CF PARTICIPANTS

Baseline assessments and postdose assessments were obtained for all subjects for both control and

drug-treated nostrils. Follow-up NPD measurements without Moli1901 were repeated weekly for at least a month and until the responses were within 20% of baseline to assess duration of drug effect.

The baseline NPD, amiloride-sensitive response, chloride-free response, and isoproterenol response for normal and CF participants prior to drug exposure (Table 1) were compared. These data show that the behavior of CF epithelia is very different from non-CF epithelia with respect to basal NPD and responses, and validate the model for biological activity on ion channels. Secondly, the chloride-free and isoproterenol responses at baseline were present in normal subjects and absent in CF participants, also consistent with previous observations.

The chloride-free, amiloride containing Ringers solution induces a hyperpolarization in normal nasal epithelia. Compound Moli1901 was administered in this solution in order to provide a driving force for chloride secretion. Figure 1 displays the NPD measurements during exposure to Moli1901 (*top panel*) and vehicle (*bottom panel*) from one of the four normal volunteers. Administration of Moli1901 resulted in transient hyperpolarizations to vehicle superfusions and escalating hyperpolarizations during exposure to drug. Data were then expressed as peak change in NPD during exposure to Moli1901 or vehicle (NPD peak value minus the NPD just prior to the dose). Moli1901 induced a significant difference in chloride transport when all doses of Moli1901 were compared with the average of all doses of vehicle (p < 0.05). Figure 2 displays the mean ± SEM of the peak change in NPD secondary to chloride efflux during each dose of drug and vehicle from four normal volunteers. A significant induction of chloride transport was present for doses of 1 μmol/L, 3 μmol/L, and 10 μmol/L when compared with the corresponding vehicle treatment (p < 0.05, paired *t* test).

Similar data for one individual from the CF group is shown in Figure 3. There are small transient repolarizations early in the dose-response curve, with significant changes visible at 3 μmol/L and 10 μmol/L. The cumulatively sustained change in

Table 1—Baseline NPD Measurements Prior to Drug or Vehicle Exposure*

Subjects	Basal NPD, mV		Δ Amiloride, mV		Δ Amiloride/chloride-free, mV		Δ Isoproterenol/chloride-free/amiloride, mV	
	Drug	Vehicle	Drug	Vehicle	Drug	Vehicle	Drug	Vehicle
Non-CF	-14.8 (3.7)	-9.6 (2.4)	3.6 (5.3)	4.8 (1.9)	-8.7 (3.7)	-15.1 (7.2)	-5.0 (7.0)	-7.2 (1.4)
CF	-35.8 (9.5)	-29.7 (8.0)	21.9 (6.8)	11.4 (8.3)	0.9 (8.2)	6.6 (5.4)	0.78 (3.3)	2.1 (4.5)

*Data are presented as mean ± SEM. Pairwise comparisons by *t* test between nares assigned to either drug or vehicle had pretreatment responses to amiloride, amiloride/chloride-free, and isoproterenol/chloride-free/amiloride solutions that were similar (p > 0.05).

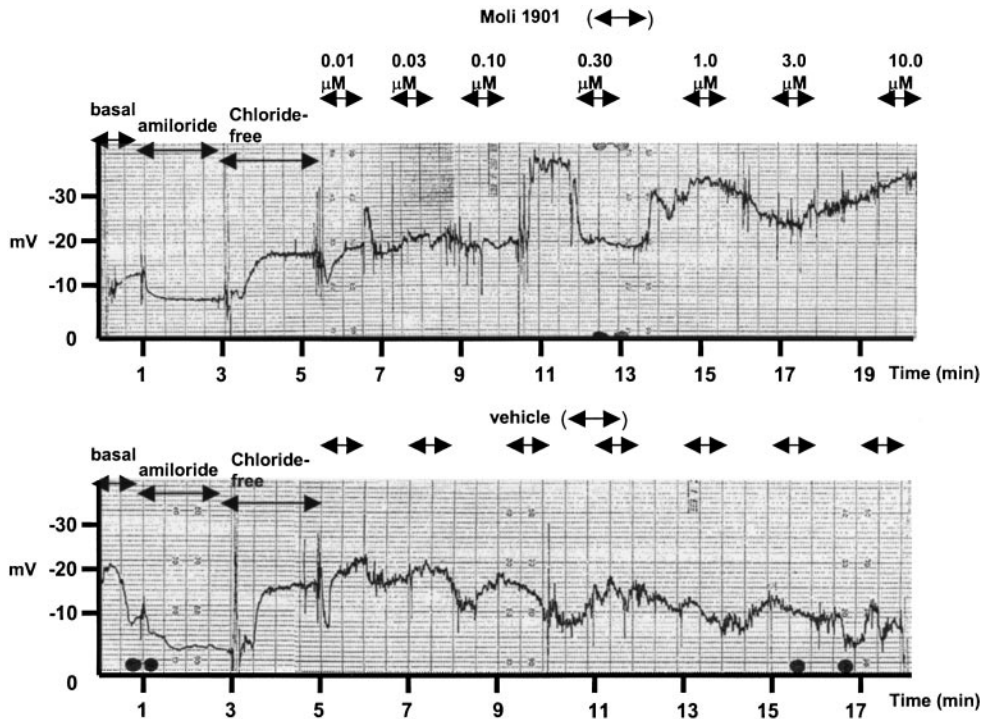


FIGURE 1. Dose escalation of Moli1901 in a non-CF participant. *Top*: Real-time NPD recording during sequential superfusions (data placed directly onto a paper recorder) of increasing doses of Moli1901. *Bottom*: Vehicle instillations. Arrows indicate the duration of each dose. At 3 min, the superfusion was switched from 100 $\mu\text{mol/L}$ amiloride in Ringers to chloride-free, gluconate-substituted Ringers in the continued presence of 100 $\mu\text{mol/L}$ amiloride. The change in NPD during this superfusion represents the 0 dose. In non-CF participants, this chloride-free solution induces chloride secretion and repolarization of the NPD.

NPD for this volunteer was approximately -11 millivolts (mV). Figure 4 contains the summary of the data from all four CF volunteers. There was more variability in dose response to Moli1901 in CF participants than in non-CF subjects. However, as a group, Moli1901 induced a significant hyperpolarization in the drug-treated nostril as an

average of all doses compared with an average of all doses of the vehicle ($p < 0.05$). A significant induction of chloride transport was observed at the dose of 3 $\mu\text{mol/L}$ when compared with the equivalent vehicle treatment ($p < 0.05$; Fig 4).

The dose-response relationships for individuals in both CF and non-CF groups were variable and might also be explained by a threshold relationship under which further dose escalations in rapid time intervals became ineffective. Determination of the ideal dosing interval may help to sustain a therapeutic effect.

NPDs were performed at weekly intervals after treatment to assess the duration of the response to Moli1901. The low chloride response was representative of the chloride transport that remains secondary to a single dose of Moli1901. Again, variability in the length of time that a persistent low chloride response could be detected in both groups was observed. Estimates for a half-life of the low chloride response could not be calculated due to small sample sizes, but a prolonged induction of the low chloride response in the drug treated naris was observed in one healthy volunteer for several weeks. The low

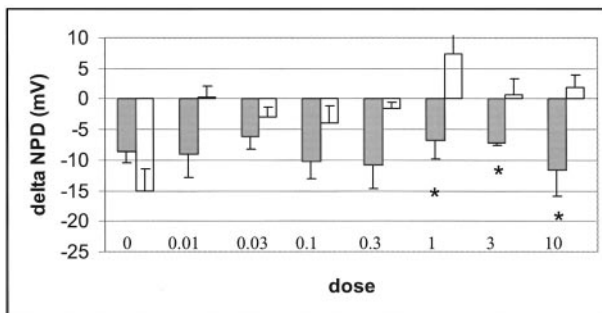


FIGURE 2. Summary of the change in NPD for healthy volunteers exposed to Moli1901 or vehicle during seven escalating doses. The data are represented by the mean \pm SEM each dose concentration of Moli1901 (solid bar) or vehicle (open bar). * $p < 0.05$ for the pairwise comparisons of drug with vehicle at each dose by t test.

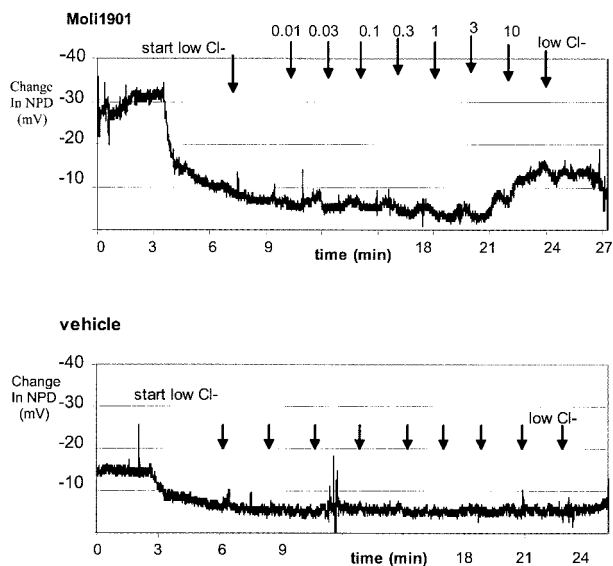


FIGURE 3. Dose escalation of Moli1901 in a participant with CF. *Top*: Real-time NPD recording (computer-based data acquisition) during sequential superfusions of increasing doses of Moli1901. *Bottom*: Vehicle instillations. Arrows indicate the duration of each dose. Amiloride (100 $\mu\text{mol/L}$ in Ringers) was added at 3 min to block the sodium potential. At 6 min, this superfusate was switched to a gluconate-substituted Ringers in the continued presence of 100 $\mu\text{mol/L}$ amiloride. This represents the 0 dose. In CF participants, this chloride-free solution does not induce chloride secretion and the potential difference typically remains unchanged or depolarizes slowly in the continued presence of amiloride.

chloride response was not as prolonged in the CF participants and was absent within a week of drug treatment.

DISCUSSION

The respiratory epithelium lining the inferior turbinate resembles structurally and functionally the

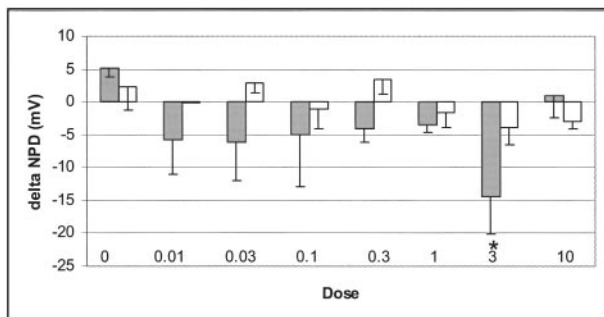


FIGURE 4. Summary of the change in NPD for CF participants exposed to Moli1901 or vehicle during seven escalating doses. As in Figure 2, the data are represented by the mean \pm SEM for each dose concentration of Moli1901 (solid bar) or vehicle (open bar). * $p < 0.05$ for the pairwise comparisons of drug with vehicle at each dose by *t* test.

epithelium lining the upper airways and can be used as a surrogate region for evaluating compounds that modulate airway epithelial ion transport.⁹ NPD measurements of basal or resting potential difference reflect the dominant sodium flux from lumen to submucosal space.^{10,11} The contribution of sodium absorption to this potential difference is estimated by superfusing with amiloride to inhibit sodium influx and measuring the change in potential difference. Inhibition of sodium absorption also allows easier detection and quantification of chloride secretion in subsequent maneuvers. When chloride is removed from the perfusate (in the continued presence of amiloride), an electrochemical gradient is induced favoring chloride movement into the luminal space and repolarization. Subsequent inclusion of agonists such as isoproterenol or the test compound Moli1901 may augment chloride secretion and further repolarization of the potential difference. NPD measurements correlate with bronchial potential difference measurements¹² supporting the use of nasal potential difference responses as a proof of principle for biological activity in the lower respiratory tract.

The effect of Moli1901 on chloride transport in normal human and CF epithelium and in canine tracheal epithelium has been studied by several investigators.^{1,2,4} Moli1901 increased short-circuit current (Isc) and net chloride secretion over a narrow concentration range,^{2,4} and had no effect on cellular integrity as measured by mannitol flux when added to the mucosal bathing solution.⁴ The maximum increase in Isc was observed at a Moli1901 concentration of 2 $\mu\text{mol/L}$, whereas higher Moli1901 concentrations produced a decrease in Isc. Pretreatment of tissues with mucosal amiloride (10⁻⁴ mol/L) to reduce basal Na⁺ transport had no effect on the subsequent response to Moli1901, making it unlikely that the effect of Moli1901 involves Na⁺ channels. In other tissues pretreated with 10⁻³ mol/L cAMP, Moli1901 produced an increase in Isc and net chloride secretion similar to its effect in nonpretreated tissues, making it unlikely that CFTR was required. Yet, the increase in Isc was entirely accounted for by an increase in net chloride secretion. Cloutier et al⁵ later demonstrated that Moli1901 stimulates intracellular calcium efflux from intracellular stores as well as promotes influx from extracellular fluid, suggesting that Moli1901 may exert its effect through activation of calcium-dependent chloride channels.

The hallmark of CF is the failure of epithelial chloride conductance secondary to absence or dysfunction of the cAMP-mediated chloride channel CFTR.¹³ Stimulation of an alternative chloride conductance, perhaps through calcium-activated chloride channels, has been proposed as a therapeutic

intervention. Experimental CF therapies utilizing apical adenosine triphosphate have demonstrated stimulation of chloride secretion through P2Y2 purinergic receptors^{9–14} via both intracellular calcium (calcium-dependent and calcium-independent) signaling pathways. Studies *in vivo* by Knowles et al⁹ suggest that calcium-activated chloride conductances are present in CF airways, but inactive unless intracellular calcium is increased. The transgenic CF knockout mouse model that lacks the classic CF pulmonary phenotype has been shown to have elevated basal levels of a calcium-activated chloride conductance.¹⁵ These data suggest that development of a medication that could elevate intracellular calcium levels and sustain chloride and fluid secretion for prolonged periods of time may be an effective way to combat the underlying pathophysiology of CF. Ideally, this medication would be administered by aerosol to allow targeting of pulmonary epithelia while minimizing systemic effects.

Preclinical studies⁵ of Moli1901 conducted in respiratory epithelial cells from both healthy humans and participants with CF suggested that Moli1901 increases calcium levels in both. An effect on fluid secretion is suggested by experiments in dogs in which Moli1901 given by aerosol increased the volume of airway surface liquid at estimated doses of 3.5 µg/kg (assuming 100% deposition).^{1,2}

The half-life of Moli1901 is prolonged; the half-life is 64 days in rat lungs.⁶ In dogs, a prolonged presence of Moli1901 resulted in a prolonged drug effect in the lungs of at least 1 week.^{2,7} Importantly, Moli1901 is minimally absorbed from the lungs of rats and dogs, and is excreted in the feces, presumably as the result of mucociliary clearance and swallowing.^{6,7} This mode of excretion may allow prolonged clinical activity in the lungs with minimal systemic side effects.

Our study was designed to detect acute and sustained changes in chloride transport as measured by NPD. The dose-response relationship appears suggestive of a threshold response in normal volunteers (Fig 2) and CF participants (Fig 4) was obtained. Possible explanations for these results include the study design in which escalating doses were applied in rapid succession over 1 min separated by 1-min intervals. The apparent lack of response at 10 µmol/L in the CF group as a whole may reflect that a maximal level had been reached allowing no further change at that dose. Given the complex mechanism of drug action, we may not have been able to distinguish acute effects from cumulative effects of repeated doses on the epithelium. Since the drug is excreted intact in the feces, it is likely that the half-life is in part determined by the half-life of the respiratory epithelium.¹⁶ Diseased

nasal epithelium as is typical in CF is likely to undergo accelerated turnover and may lead to a shorter half-life for Moli1901. Interestingly single doses of aerosolized Moli1901 achieving 30 µg/kg in non-CF and CF participants have now been shown to be safe (personal communication, March 2002; Luis Molina).

CONCLUSIONS

In conclusion, Moli1901 was safe and well tolerated in nasal epithelium exposed to a concentration of up to 10 µmol/L. Chloride secretion was induced at 1 µmol/L, 3 µmol/L, and 10 µM in normal volunteers. CF participants also generated chloride secretion in response to Moli1901 at 3 µM, but more variability was observed. Further, these studies justify the conduct of a pilot efficacy study of Moli1901 in CF.

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